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Research Article

# A STABILITY STUDY OF AYURVEDIC LAXATIVE FORMULATION - CONSTAC GRANULATION

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**ABSTRACT**

Nowadays herbal medicines are accepted all over the world. The major weakness is consistency in the quality of the product over a range of time. So stability testing is necessary to ensure the quality of herbal products which is an evidence for the quality of the finished product. The present study was conducted to evaluate accelerated stability (Temperature: 40 °C ± 2, Relative Humidity (RH): 75% ± 5) and real time stability (Temperature: 25 °C ± 2, Relative Humidity (RH): 60% ± 5) of Constac granulation. A study was conducted as per ICH guideline Q1A (R2). The variation in the organoleptic, physico-chemical and microbial load constants of the Constac granulation (fine granulation) was observed during 0 (initial), 1st, 3rd and 6th month. Results of different physico-chemical parameters were taken into consideration to evaluate intercept and slope. It was found that there was no change in color, odour and taste of Constac granulation up to storage of 6 months at accelerated condition. Constac granulation was suitable at accelerated condition up to 6month storage. It can be extrapolated that shelf life of Constac granulation is 23.02 months. The real time stability data of Constac granulation showed very good stability up to 1 year.

**KEYWORDS:** Accelerated stability, Real time Stability, Constac Granulation.

## INTRODUCTION

Currently herbal medicines are accepted all over the world. The Major weakness is consistency in the quality of the product over a range of time.[1] Any considerable changes in the quality of the product over a time must be detectable. So stability testing is needed to ensure the quality of herbal products which is an evidence for the quality of the finished product.[2] The products efficacy, safety, and ethical issue have to be confirmed before launching in the market. Herbal medicinal products have to fulfill the legal requirements with regard to quality, including stability testing, but have certain particularities such as a complex nature, an often low concentration of constituents and a natural variability of their raw materials.[3] Due to their natural origin, questions on microbiological quality arise more often for herbal medicinal products than for chemically defined medicinal products.[4] Hence stability testing is essential to improve the quality of the herbal products. According to Ayurvedic pharmaceutical science, *Churna* preparations remain potent up to two months, after which they start degrading gradually losing their efficacy.[5] There are two types of stability study one is accelerated stability and other is real time stability. Pharmaceutical products are generally studied for stability profile at accelerated temperature and humidity, the experimental findings of which can be very helpful to predict reliable self-life or expiry date at room temperature by adopting certain assumptions and criterions. [6] In the present study stability study was carried out to determine shelf-life of Constac granulation.

## MATERIAL AND METHODS

### Test drug- Constac granulation

CONSTAC (Figure 1, 2) is an Ayurvedic proprietary polyherbal formulation in granulation form which comprises dried granulations of *Terminalia chebula, Terminalia Belerica, Emblica officinalis, Plantago Ovata, Glycyrhiza glabra, Ptychotis Ajowan, Foeniculum Vulgare, Elliteria Cardamomum, Coscos Nucifera, Coscos Nucifera.* All the ingredients of the formulation have been used for thousands of years and individual therapeutic efficacy of these herbs as laxative has also been reported in an ancient Ayurvedic literature.[7,8] A freshly prepared Constac granulation was considered for stability study. Constac granulation was packed in airtight food grade plastic container having aluminum foil covering.

Figure 1: Constac granulation


Figure 2: Constac product

**Storage condition and evaluation parameters:** Accelerated stability study and real time stability study was conducted as per ICH guideline Q1. A(R2).[9] Storage condition are mentioned as below,

- Accelerated stability: Temperature: 40 °C ± 2, Relative Humidity (RH): 75 % ± 5
- Real time stability: Temperature: 25 °C ± 2, Relative Humidity (RH): 60 % ± 5

The change was observed during 6 months for accelerated stability and 1 year for real time stability study at an interval of 0,1,3,6 and 12 months. Real time stability was comparatively carried out to evaluate the actual degradation rate of Constac granulation with respect to accelerated condition. 10% degradation was set to extrapolate of the accelerated stability data at the acceptable point. Real time aging factor 5 and 3.3 were used for extrapolation of shelf life.

The following parameters were considered for evaluation of stability study.

- Organoleptic characters like colour, odour and taste
- Physico-chemical parameters like Loss on drying, pH, Total ash, Water soluble extractive value, bitter residue, total saponin and total tannin.
- Microbial load

**Organoleptic analysis**

The colour indicated the nature of the formulation; odor and taste of the formulation are extremely sensitive criteria.

**Color:** 5 gram Constac granulation was taken into watch glasses and placed against white background in white tube light and was examined for their color by naked eye.

**Odour:** 2 gram Constac granulation was smelled.

**Taste:** A pinch of Constac granulation was taken and examined for it's taste on taste buds of the tongue.

**Loss of drying**

Loss on drying was determined by weighing about 2gm of the granulation ed material in previously weighed dried petridish (tarred evaporating dish) and dried in an oven at 105ºC, till two consecutive weights, which do not differ by more than 5mg. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as % w/w with reference to air dried sample. [10]

**pH**

1 g of Constac granulation in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

**Water soluble extractive value**

About 5 g accurately weighed Constac granulation was macerated in a glass-stopper conical flask. 100 ml chloroform water was added and macerated for 6 h, shaking frequently and then allowed to stand for 18 h then after 24 hrs it was filtered rapidly and 20 ml of the filtrate was transferred in a tarred flat bottom evaporating dish with a pipette and evaporated to dryness on a boiling water bath. Then evaporating dish was dried at 105ºC for 6 h and then cooled and weighed. From the weight of the residue the percentage of water soluble extractive was calculated and expressed as % w/w with reference to air dried sample. [10]

**Total ash**

The ash value was determined by incinerating about 5g of the granulation air-dried material, in a previously weighed crucible at gradually increasing heat up to 450 ºC until it is carbon free. Then cooled in a desiccator and weighed. The percentage of total ash was calculated and expressed as % w/w of air dried material.[10]

**Total Tannin**

For blank preparation: 300 ml of distilled water was taken in a 500 ml conical flask. 25 ml of indigo sulphonic acid solution was added and mix well. Titrated against 0.02M KMnO4 solution till stable golden yellow color was developed. The burette reading was noted.

For sample preparation: Accurately weighed about 0.05 g of the sample. Material was transferred to a 500 ml conical flask. To it 50 ml of distilled water was added and mixed well to dissolve completely. To this 250 ml of distilled water was added and mixed well, then sonicate it for 10 min. 25 ml of the indigo sulphonic acid solution was added and mixed well. Titrated against 0.02M KMnO4 solution till stable golden yellow color was developed. The burette reading was noted. The percentage of total tannin was calculated using following factor. 1 ml of 0.02M KMnO4 is equivalent to 0.00415g of tannin substance.[11]

**Bitter residue**

One gram of the test material was taken in a 150 ml conical flask. To it 50 ml of methanol was added. It was refluxed for half an hour on a water bath. Then filtered and collected the methanol extract in a

250 ml beaker. The residue was extracted for another two cycles of extraction. Three (or all the extracts if greater than 3) methanol extracts were pooled and evaporated it to obtain a thick paste (not free flowing) approximate 5 ml volume. Now shake the concentrated extract with three successive cycles of 25 ml hot water or till all the water soluble matter is extracted or dissolved. Above three (or greater than 3) water washed extracts were pooled and transferred it to a separating funnel. This aqueous extract was extracted with minimum 4 cycles of 25 ml of petroleum ether (60-80 0C). Then extract the petroleum ether washed aqueous extract with 25 ml of ethyl acetate. Ethyl acetate extraction was repeated for another three more cycles. The ethyl acetate extracts were pooled and transferred to a pre-weighed evaporating dish and evaporated to dryness. From the weight of the residue the percentage of bitter residue was calculated and expressed as % w/w with reference to air dried sample.[12]

**Estimation of Total Saponin**

Five grams of test material were weighed in a conical flask. To this 50 ml 90%v/v methanol was added. Content was mixed well and refluxed for half an hour. After cooling it was filtered. Wash the residue with 90%v/v methanol till washing were almost colorless. Methanol extract was combined and evaporated on a water bath to obtain a thick paste like residue. The residue was treated with 25 ml petroleum ether (60-80 0C). Petroleum ether layer was separated and discarded. The residue was treated with 25 ml chloroform. Chloroform layer was separated and discarded. The residue was treated with 25 ml ethyl acetate. Ethyl acetate layer was separated and discarded. Then 5 ml 90%v/v methanol was added in residue. Content was shake well to dissolve the residue completely. Now pour this solution drop wise with constant stirring into a beaker containing 25 ml acetone to obtain precipitate. Rinse the flask containing the residue with minimum volume (about 2 ml) of 90%v/v methanol. Decant the organic layer and dry the residue to constant weight. The percentage of total saponin was calculated and expressed as % w/w with reference to air dried sample

**Microbial load**

Microbial load was conducted as per standard procedure mentioned in Indian Pharmacopoeia. It included a total bacterial count, total Fungal Count, presence of Escherichia coli, Salmonella spices, Pseudomonas aeruginosa and Staphylococcus aureus.

All tests were done by Shri Bhaurao Patil collage of bio-informatics & IT Hingoli Pune.

**RESULTS**

In the accelerated stability study, Temperature: 40°C ± 2, Relative Humidity (RH): 75% ± 5 was maintained up to 6 months. The product was analyzed on 0, 1, 3 and 6 months. No change was observed in color, odour and taste of formulation up to storage of 6 months at accelerated condition (Table 1). Results of microbial load of Constac granulation was complying with Ayurvedic Pharmacopeial limits at initial month and up to 6 months (Table 1). Results of different physicochemical parameters were taken in consideration to evaluate intercept and slope (Table 2). The extrapolated shelf life of Constac granulation was calculated with 10% degradation rate from physicochemical parameters at accelerated condition 40°C ± 2 and 75% ± 5 RH (Table 3).

**Table 1: Results of different parameters of Constac granulation at 40 C +/- 2 C and 75% +/- 5 RH in different interval**

| Parameters | Initial Month | 1st month | 3rd month | 6th month |
|---|---|---|---|---|
| Colour | Brown Colored Granulation | Complies | Complies | Complies |
| Odour | Characteristic | Complies | Complies | Complies |
| Taste | Bitter and salty | Complies | Complies | Complies |
| Loss on drying(% w/w) | 3.58 | 4.24 | 4.95 | 5.89 |
| pH value(1%w/v solution) | 5.6 | 5.9 | 6.2 | 6.5 |
| Total Ash(% w/w) | 7.68% | 7.36% | 6.94% | 6.59% |
| Water Soluble Extractive Value (%w/w) | 38.192% | 30.72% | 30.72% | 29.736 % |
| Alcohol Soluble Extractive Value (%w/w) | 4.128% | 4.032% | 4.032% | 4.026% |
| Bitter residue (%w/w) | 3.89 | 3.68 | 3.29 | 3.15 |
| Total Saponin (%w/w) | Present | Present | Present | Present |
| Total Tannin (%w/w) | Present | Present | Present | Present |
| Total Bacterial Count | $30x10^3$ | $50x10^3$ | $26x10^4$ | $35x10^5$ |
| Total yeast & mould | $18x10^1$ | $24x10^1$ | $40x10^2$ | $75x10^3$ |
| *E.coli* | Absent | Absent | Absent | Absent |
| *P.areuginosa* | Absent | Absent | Absent | Absent |
| *S.aureus* | Absent | Absent | Absent | Absent |
| *S.spp* | Absent | Absent | Absent | Absent |

**Table 2: Intercept and slope of different physio chemical parameters of Constac granulation**

| Parameters | Initial Month | 1st month | 3rd month | 6th month | Slope | Intercept |
|---|---|---|---|---|---|---|
| Loss on drying (% w/w) | 3.58 | 4.24 | 4.95 | 5.89 | 0.37 | 3.74 |
| pH value | 5.6 | 5.9 | 6.2 | 6.5 | 0.14 | 5.69 |
| Total Ash(% w/w) | 7.68% | 7.36% | 6.94% | 6.59% | 0.18 | 7.58 |
| Water Soluble Extractive Value (%w/w) | 34.72% | 32.16% | 31.02% | 33.264 % | 0.147 | 33.161 |
| Bitter residue (%w/w) | 3.89 | 3.68 | 3.29 | 3.15 | 0.12 | 3.80 |
| Total Saponin (%w/w) | Present | Present | Present | Present | | |
| Total Tannin (%w/w) | Present | Present | Present | Present | | |

**Table 3: Extrapolated shelf life of Constac granulation from different physiochemical parameters**

| Parameters | Initial Month Results | Results at 10% degradation | Months when 10% degradation occurs |
|---|---|---|---|
| Loss on drying(% w/w) | 3.58% | 3.23% | 1.4 |
| pH value | 5.6 | 4.62 | 4.6 |
| Total Ash(% w/w) | 7.68% | 6.92% | 3.7 |
| Water Soluble Extractive Value (%w/w) | 34.72% | 31.248% | 13 |
| Bitter residue (%w/w) | 3.89% | 3.51% | 2.4 |
| Total Saponin (%w/w) | Present | Present | |
| Total Tannin (%w/w) | Present | Present | |
| Mean Months at accelerated condition | | | 5.02 |
| Extrapolated shelf life of Constac granulation | | | 23.02 months |

## DISCUSSION

Stability testing is necessary to ensure the quality of an herbal product. The main objective of stability testing of pharmaceutical products is to ensure the efficacy and quality of active compounds in product, to establish shelf life or expiration period and to support the label claim. As per Drug and Cosmetic act, the optimal climatic condition for the storage of medicine is 25 °C/60% RH. If we can maintain same climatic condition for the storage of Constac granulation then shelf life of Constac granulation is near to 23.02 months. It is matched with the implemented rule namely 161 B to display the date of expiry of the ASU drugs and propose shelf life of Ayurvedic formulations i.e. shelf life of *Churna* (fine / course granulation drugs) as 2 years.

## CONCLUSION

Study results support the formulation was suitable at accelerated condition up to 6 month storage and real time stability data showed very good stability up to 1 year.

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